# Drug resistant clones of the AS lineage

## 2. Published papers

### Early preparation

Walliker, Carter and Morgan (1971) Genetic recombination in malaria parasites. *Nature* **232** 561-562

*First indication of genetic recombination using rodent malaria (*P. yoelii *17X and 33X*). *Starch chromatograms and alleles of glucose phosphate isomerase*

Walliker, Carter and Sanderson (1971) Genetic studies on *Plasmodium chabaudi*: recombination between enzyme markers. *Parasitology* **70** 19-24

*First demonstration of genetic recombination using* P. chabaudi *rodent malaria strains AS-PYR and AJ (using alleles of 6-phosphocluconate dehydrogenase and lactate dehydrogenase as markers)*

### Drug resistance, generation of phenotype, mapping and sequencing of genetic determinants

Rosario (1976) Genetics of chloroquine resistance in malaria parasites. *Nature* **261** 585-586

*Generation (selection) and cloning of AS-3CQ. Cross with AJ and demonstration of recombination of LDH, 6PGD genotypes and PYR and CQ phenotypes.*

Padua (1981) *Plasmodium chabaudi*: Genetics of resistance to chloroquine. *Experimental Parasitology* **52** 419-426

*Generation of AS-15CQ and AS-30CQ, cloning of AS-30CQ. Crossing of parasites with AJ and recombination of genetic markers and PYR and CQ phenotypes. Note that "AS-0CQ" is alternative terminology for AS-PYR*

Carlton, Mackinnon and Walliker (1998) A chloroquine resistance locus in the rodent malaria parasite *Plasmodium chabaudi*. *Molecular Biochemical Parasitology* **93** 57-72

*Analysis of cross AS-3CQ x AJ. Linkage analysis using ~ 20 clones and 45 genetic markers (RFLPs). Genetic map. QTL analysis. Map principal locus to chr11 (and very tentative associations at loci on chr03, 05 and 09).*

Hayton, Ranford-Cartwright and Walliker (2002) Sulfadoxine-Pyrimethamine resistance in the rodent malaria parasite *Plasmodium chabaudi*. *Antimicrobial Agents Chemotherapy* **46** 2482-2489

*Generation and cloning of AS-50S/P (sulfadoxine and pyrimethamine resistant) from AS-PYR. Cross with AJ. Linkage analysis and QTL. Identification of chr07 as principal locus for PYR, SDX (negative) and SDX/PYR and locus on chr13 as principle locus for SDX (positive). Incorrect/unreliable re sequencing of AJ* dhfr.

Cravo, Carlton, Hunt, Bisoni, Padua and Walliker (2003) Genetics of mefloquine resistance in the rodent malaria parasite *Plasmodium chabaudi*. *Antimicrobial Agents Chemotherapy* **47** 709-718

*Generation and cloning of AS-15MF (mefloquine resistant) from AS-15CQ. Cross with AJ. Linkage analysis. Identification of mdr1 duplication and linkage with MF-R phenotype*

Hunt, Cravo, Donleavy, Carlton and Walliker (2004a) Chloroquine resistance in *Plasmodium chabaudi*: are chloroquine-resistance transporter (*crt*) and multi-drug resistance (*mdr1*) orthologues involved? *Molecular Biochemical Parasitology* **133** 27-35

*Characterisation (sequencing) of* crt *orthologue, Linkage analysis of* crt *and* mdr1 *alleles in recombinant clones of AS-3CQ cross and AS-15MF cross. No linkage with CQ-R phenotype*

Hunt, Martinelli, Fawcett, Carlton, Carter and Walliker (2004b) Gene synteny and chloroquine resistance in *Plasmodium chabaudi*. *Molecular Biochemical Parasitology* **136** 157-164

*Linkage analysis of AS-3CQ* x *AJ cross and AS-30CQ* x *AJ cross using 658 AFLP markers. Mapped CQ-R locus to 250kb on chr11. Demonstration of synteny relative to* P. falciparum

Afonso, Hunt, Cheesman, Alves, Cunha, Rosario and Cravo (2006) Malaria parasites can develop stable resistance to artemisinin but lack mutations in candidate genes *atp6*, *tctp*, *mdr1* and *cg10*. *Molecular Biochemical Parasitology* **133** 27-35

*Generation and cloning of AS-ART and AS-ATN. Artemisinin and artesunate responses. No mutations regarding sequencing of* atp6, tctp, mdr1 and cg10/crt. *Unreliable regarding artemisinin and artesunate responses (see Hunt et al 2010)*

Hunt, Afonso, Creasey, Culleton, Sidhu, Logan, Valderramos, McNae, Cheesman, Rosario, Carter, Fidock and Cravo (2007) Gene encoding a deubiquitinating enzyme is mutated in artesunate- and chloroquine-resistant rodent malaria parasites. *Molecular Microbiology* **65** 27-40

*Identification of two different mutations in* ubp1*, namely V739F in AS-ATN and V770F in AS-30CQ and AS-ART relative to AS-3CQ. Note that these mutations are renumbered in subsequent publications as V2697F and V2728F after re-annotation of the* ubp1 *gene in more recent assemblies of AS genome. Cross between AS-ART and AJ. First evidence (weak) of selection on chr02 (locus of* ubp1*) following artemisinin treatment using Linkage Group Selection paradigm. Mapping of mutations to active site/Ub binding pocket.*

Rodrigues, Henriques, Borges, Hunt, Sanchez, Martinelli and Cravo (2010) Experimental Evolution of resistance to artemisinin combination therapy results in amplification of the *mdr1* gene in a rodent malaria parasite. PLoS ONE **5** e11593

*Generation and cloning of AS-ATNMF1 after artesunate and mefloquine selection from AS-ATN. Identification of amplification and over-expression of* mdr1

Hunt, Martinelli, Modrzynska, Borges, Creasey, Rodrigues, Beraldi, Loewe, Fawcett, Kumar, Thomson, Trivedi, Otto, Pain, Blaxter, Cravo (2010) Experimental evolution, genetic analysis and genome re-sequencing reveal the mutation conferring artemisinin resistance in an isogenic lineage of malaria parasites *BMC Genomics* **11** e499

*Defined* ubp1 *mutations as those conferring artemisinin resistance. ART-R phenotype defined in AS-30CQ, AS-15MF, AS-ATN and AS-ART (partial correction of Afonso* et al*. 2006). LGS analysis (ART treatment) of AS-30CQ* x *AJ and the AS-15MF* x *AJ crosses (backcrosses) identified selection valley in middle of chr02. Genome-wide re-sequencing (Illumina) confirmed one mutation only on chr02* (ubp1)*. Also confirmed only 4 point mutations shared between AS-15MF and AS-30CQ relative to AS-sens. These included V2728F* ubp1 (*appearing first in AS-15MF and AS-30CQ*) *and S106N* dhfr (*appearing first in AS-PYR1*). *The other mutations are fully identified in future papers.*

Martinelli, Henriques, Cravo and Hunt (2011) Whole genome re-sequencing identifies a mutation in an ABC transporter (*mdr2*) in a *Plasmodium chabaudi* clone with altered susceptibility to antifolate drugs. *International Journal for Parasitology* **41** 165-171

*Genome re-sequencing of AS-50S/P. Identifies 2 point mutations in AS-PYR1 (relative to AS-sens) including S106N* dhfr *and a non-coding mutation on chr14. There was also a 34 bp deletion on chr07. In AS-50S/P relative to AS-PYR1 there were two additional point mutations. One of these was K392Q* mdr2 *on chr13. This maps between two markers most closely associated in SDX response QTL analysis (Hayton* et al. *2002, above*).

Borges, Cravo, Creasey, Fawcett, Modrzynska, Rodrigues, Martinelli and Hunt. (2011) Genome-wide scans reveals amplification of mdr1 as a common denominator of resistance to mefloquine, lumefantrine and artemisinin in *Plasmodium chabaudi* malaria parasites. *Antimicrobial Agents and Chemotherapy* **55** 4858-4865

*LGS analysis of AS-15MF* x *AJ cross selected with mefloquine, lumefantrine and artemisin selection shows selection on* mdr1 *duplication (chr04, chr12). Full Illumina analysis of genome re-sequencing, copy number analysis of* mdr1*, analysis and confirmation of* mdr1 *translocation event*.

Modrzynska, Creasey, Loewe, Cezard, Borges, Martinelli, Rodrigues, Cravo, Blaxter, Carter, Hunt (2012) Quantitative genome re-sequencing defines multiple mutations conferring chloroquine resistance in rodent malaria. *BMC Genomics* **13** e106

*Characterises CQ-R and CQ-hiR phenotypes. LGS analysis AS-30CQ* x *AJ backcross at multiple CQ doses identifies selection valleys on chr11, chr03 and chr02. Genome re-sequencing identifies the mutations at these loci causing CQ-R, intermediate and high resistance. These are A173E* aat1 (*aminoacid transporter, chr11*)*, two mutations on chr03 and V2728F* ubp1 *on chr02*

Henriques, Martinelli, Rodrigues, Modrzynska, Fawcett, Houston, Borges, d'Alessandro, Tinto, Karema, Hunt and Cravo (2013) Artemisinin resistance in rodent malaria – mutation in the AP2 adaptor μ-chain suggests involvement of endocytosis and membrane protein trafficking. *Malaria Journal* **133** 27-35

*Characterisation of increased ART-R phenotype in AS-ART (relative to AS-30CQ). Genome re-sequencing of AS-ART reveals one point mutation (relative to AS-30CQ), I568T in AP2 mu-chain (chr14), implicating clathrin-mediated endocytosis in resistance mechanism. Mutation maps to residue interacting with YXXΦ recognition sequence on cargo.*

## Genetic markers, novel genetic mapping (LGS) strategies, genetic map

Grech, Martinelli, Pathirana, Walliker, Hunt and Carter (2002) Numerous, robust genetic markers for *Plasmodium chabaudi* by the method of Amplified Fragment Length Polymorphism. *Molecular Biochemical Parasitology* **123** 95-104

*Generation and characterisation of >600 AFLPs (AJ v AS) for mapping.*

Culleton, Martinelli, Hunt and Carter. (2005) Linkage group selection: Rapid gene discovery in malaria parasites. *Genome Research* **15** 92-97

*Demonstration of LGS, using pyrimethamine resistance as a model system..*

Martinelli, Cheesman, Hunt, Raza, Mackinnon and Carter. (2005a) A genetic approach to the *de novo* identification of targets of strain-specific immunity in malaria parasites. *Proc. Natl. Acad. Sci. (USA)* **102** 814-819

*Demonstration of LGS for strain-specific immunity* (*showing selection on* msp1)*.*

Martinelli, Hunt, Fawcett, Cravo, Walliker and Carter. (2005b) An AFLP-based Genetic Linkage Map of *Plasmodium chabaudi chabaudi*. *Malaria Journal* **4** 11 -20

*Genetic map of AFLP markers*

Carter, Hunt, and Cheesman. (2007) Linkage Group Selection - a fast approach to the genetic analysis of malaria parasites. *International Journal Parasitology* **37:** 285-293

*Review article explaining principles of Linkage Group Selection.*

## Quantitation of allele frequency (LGS depends on quantitation of genetic marker intensity)

Martinelli, Hunt, Cheesman and Carter (2004) Amplified Fragment Length Polymorphism measures proportions of malaria parasites carrying specific alleles in complex genetic mixtures. *Molecular Biochemical Parasitology* **136** 117-122

*Quantitation of AFLP markers by densitometry.*

Hunt, Fawcett, Carter and Walliker. (2005) Estimating SNP proportions in populations of malaria parasites by sequencing; validation and applications. *Molecular Biochemical Parasitology* **143** 173-182

*Quantitation of SNPs by sequencing.*

Cheesman, Creasey, Degnan, Afonso, Cravo, Carter and Hunt. (2007) Validation of pyrosequencing for accurate and high throughput estimation of allele frequencies in malaria parasites. *Molecular Biochemical Parasitology* **152:** 213-219

*Quantitation of SNPs by pyrosequencing.*

Modrzynska, Creasey, Loewe, Cezard, Borges, Martinelli, Rodrigues, Cravo, Blaxter, Carter, Hunt (2012) Quantitative genome re-sequencing defines multiple mutations conferring chloroquine resistance in rodent malaria. *BMC Genomics* **13** 106

*Quantitation of SNPs using Illumina sequencing.*